In the Search for New Anticancer Drugs, XVI
Selective Protection and Deprotection of Primary Amino Groups
in Spermine, Spermidine and Other Polyamines

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Spermine, Spermidine, Polyamines, Nefkens Reagent, N-Ethoxycarbonylphtalalimide

Spermidine, spermine and other polyamines 1–5 were selectively protected at the terminal primary amino functions without affecting the secondary amino groups using N-ethoxycarbonylphthalimide (15), the Nefkens' reagent. Three representative products, 17, 18 and 20, readily underwent acylation at the secondary amino nitrogen to give the corresponding compounds 21–26. Selective deprotection of two representative samples 22 and 25 at the primary amino function by hydrazinolysis yielded the corresponding derivatives 27 and 28 with free primary amino groups.

In summary, the application of Nefkens' reagent for the terminal protection of primary amino groups in various polyamines results in a simple, efficient and selective one-step procedure using commercially available reagents.

Introduction

In the past two decades considerable efforts have been expanded to the elucidation of structures and biological functions of various naturally occurring polyamines [1–4]. The elevated levels of polyamines in cancer patients have been correlated with the rate of proliferation of cancer cells [5]. On the basis of these results it was proposed [6] to use polyamines as cancer markers. Furthermore, attempts have been made to suppress the formation of polyamines by the administration of suitable inhibitors of ornithine decarboxylase which is responsible for the formation of putrescine which, in turn, is considered to be the key intermediate in the formation of other polyamines [7, 8]. With this approach, it was hoped to retard the proliferation of cancer cells and hence to develop a chemotherapy for certain cancers.

Another interesting approach was proposed [9] for the design of antineoplastic drugs by suitable de-

\[
H_2N-(CH_2)_n \cdot NH-(CH_2)_m \cdot NH_2
\]

1: n = 3, m = 4
2: n = m = 3
3: n = m = 2

\[
H_2N-(CH_2)_n \cdot NH-(CH_2)_m \cdot NH-(CH_2)_n \cdot NH_2
\]

4: n = 3, m = 4
5: n = m = 2

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In summary, the application of Nefkens' reagent for the terminal protection of primary amino groups in various polyamines results in a simple, efficient and selective one-step procedure using commercially available reagents.
rily for the introduction of acyl groups at the N\textsuperscript{1}-position of spermidine, and, consequently, is of limited value for a general preparation of the desired terminally protected polyamine derivatives.

In the third method [14] the reaction of spermidine with formaldehyde produces 1-(4-aminobutyl)-hexahydropyrimidine (8) which on successive acylation and ring cleavage is transformed into terminally N-acylated polyamine derivatives (9). However, this procedure apparently cannot be adopted for homospermidine, and the ring cleavage conditions might be a limiting factor [9].

More recently, a comprehensive methodology was developed [9] for the N-acylation of terminal primary amino groups of triamines via their N-benzylated derivatives at the secondary nitrogen atom (10). The necessary N-benzylated compounds (10) are readily synthesized [15, 16] from the commercially available benzylamine and 3-(benzylamino)-propionitrile. Although this methodology is convenient and relatively simple, it nevertheless, requires a minimum of four steps to secure the desired, selectively protected polyamine derivatives (12). Furthermore, an adaptation of this methodology to the protection of linear tetramines, such as spermine, has not been reported.

The most recently reported methodology [17] appears to obviate these problems. In this method, 1,3-thiazolidine-2-thione is used as the transfer agent for the protecting moieties. Thus, the reaction of 3-benzyloxy carbonyl-1,3-thiazolidine-2-thione (13, \( R = C_6H_5CH_2O^- \)) with spermidine (1) gave the \( N^1,N^{10}\)-bis(benzyloxy carbonyl)spermidine (9, \( R = C_6H_5CH_2O^- \)) in 69% yield [17], and the reaction of various 3-acyl-1,3-thiazolidine-2-thiones (13) with spermidine (1) and spermine (4) rendered the corresponding terminally N-acylated polyamine derivatives (9) and (14) [18–21].

It was suggested [19] that the high selectivity achieved with this methodology is attributable to hydrogen bonding involving the hydrogen of the amide moiety and the lone pair of electrons of the secondary amino group, thus decreasing the nucleophilicity of the secondary amino group.

However, the same hydrogen bond formation responsible for the good selectivity may also cause [17] difficulties during the subsequent acylation step of the secondary amino group in terminally protected polyamines.
These difficulties were eventually resolved [17] by the use of either the phenyl-bis(2-thiono-1,3-thiazolidine-3-yl)phosphine oxide reagent which is commercially not available or triphenylphosphine/2,2'-dipyridyl-disulfide reagents [22–24] which resulted in lower yields of products.

In summary, all presently known methods suffer from some drawbacks. Ideally, a selective reagent for the protection of terminal primary amino groups should be (1) readily commercially available, (2) should be inexpensive, and (3) the protecting reaction should involve one step without involvement of the secondary amino group which would be available for further direct derivatization.

In our ongoing research on new anticancer drugs we have also been interested in derivatization of spermine and spermidine at secondary amino groups. Since the existing methodologies appeared to be unsatisfactory for our purposes which could involve sensitive moieties it was decided to search for a new approach for the protection of primary amino functions without affecting the secondary amino groups.

**Results and Discussion**

Now we have developed a convenient, selective, one-step method for the protection of the primary amino functions using N-ethoxycarbonylphthalimide (15) introduced by Nefkens [25] about 25 years ago. Although this reagent has been widely used for the N-protection of amino acids [25, 26], to the best of our knowledge, no application of this reagent for the selective protection of primary amino groups in poly-amines has been reported. The to date reported diethylenetriamine and triethylenetetramine derivatives protected with the phthaloyl moiety at the terminal amino groups have been prepared using either some difficult to obtain intermediates or harsh experimental conditions [27, 28]. These methodologies were, therefore, of limited value in our studies.

In our approach the one-step reaction of five representative polyamines 1–5, including the naturally occurring spermidine (1) and spermine (4), with the Nefkens’ reagent (15) resulted in the production of the corresponding terminally N-protected derivatives 21–25 in moderate to good yields of 53–86%.

In practice, compounds 21–25 were conveniently synthesized in 45 min at room temperature by mixing the dichloromethane solutions of two molar equivalents of 15 with one molar equivalent of 1–5. In order to ascertain the preferred reactivity of 15 with primary amino groups as compared to the secondary amino groups, three equivalents of 15 were interacted with one equivalent of 3 in chloroform at room temperature for 15 min and at 60 °C for 30 min. However, in spite of an excess of 15, only one product (18) was isolated.

In all experiments the purification of the less soluble products in methanol was achieved by triturating with methanol, and the more soluble products were purified by recrystallization from methanol.

The melting point of compound 20 (m.p. = 153–155 °C) was significantly different from that (m.p. = 233–234 °C) found in the literature [18]. Since our comprehensive analytical data strongly support the proposed structure of 20 it is concluded that the previously reported compound is not identical with our product (20).
The terminally N-protected phthaloylated polyamines readily undergo acylation reactions at the secondary amino nitrogen. Thus, for example, the reaction of compounds 18 and 20 with benzoyl chloride in the presence of triethylamine gave derivatives 22 and 26 in 73% and 92% yield, respectively. Analogously, the reactions of 17 with valeryl and glutaryl chlorides gave 21 and 25 in 80% and 82% yield, respectively. Similarly, the acylation of 18 with succinic anhydride in the presence of pyridine readily produced the 2-carboxyethyl derivative 24 in 88% yield, and the reaction of 18 with phenylisocyanate resulted in the urea derivative 23 in 97% yield.

The selective deblocking of primary amino groups without affecting the secondary amino functions can be achieved either by the hydrazinolysis [29, 30] or aminolysis [31] with a 40% aqueous methylamine solution. Whereas the use of the latter reagent was unsuccessful, the hydrazinolysis of two selected samples 22 and 25 in boiling ethanol afforded products 27 and 28 which were isolated as their oxalic acid salts in 86% and 67% yield, respectively. All products were characterized by microanalysis and IR, \(^1\)H NMR, \(^13\)C NMR and mass spectrometries (Table I). The purity control of all products was also monitored by the TLC analysis.
Table I. \(N^a,N^o\)-Bis(phthaloyl)polyamines 16–20.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield [%]</th>
<th>m.p. [°C]</th>
<th>Molecular formula</th>
<th>M.S.</th>
<th>IR (KBr)</th>
<th>(^1)H NMR (CDCl(_3))</th>
<th>(^{13})C NMR (CDCl(_3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>75</td>
<td>143–145</td>
<td>(C_{23}H_{23}N_4O_4)</td>
<td>405.43</td>
<td>406(100)</td>
<td>1026, 1362, 1394, 1437, 1466, 1718, 1769, 2932, 3227</td>
<td>1.05–1.85 (m, 6H); 26.37; 27.36; 28.90; 2.40 (t, 4H); 3.22–3.60 (m, 4H); 6.90–7.30 (m, 8H)</td>
</tr>
<tr>
<td>17</td>
<td>86</td>
<td>134–135</td>
<td>(C_{22}H_{24}N_4O_4)</td>
<td>391.41</td>
<td>392(100)</td>
<td>1047, 1396, 1435, 1466, 1770, 2946, 3330, 3361</td>
<td>1.38–1.95 (m, 5H); 2.43 (t, 4H); 3.45 (t, 4H); 6.90–7.35 (m, 8H)</td>
</tr>
<tr>
<td>18</td>
<td>82</td>
<td>178–180</td>
<td>(C_{21}H_{24}N_4O_4)</td>
<td>364(100)</td>
<td>365(24)</td>
<td>1047, 1372, 1435, 1466, 1713, 2946, 3225</td>
<td>2.70 (t, 4H); 3.47 (t, 4H); 7.07 (b.s., 8H)</td>
</tr>
<tr>
<td>19</td>
<td>61</td>
<td>91–93</td>
<td>(C_{20}H_{24}N_4O_4)</td>
<td>463(100)</td>
<td>464(24)</td>
<td>1343, 1384, 1439, 1467, 1707, 1767, 2942</td>
<td>1.16–2.13 (m, 10H); 27.94; 29.01; 36.02; 2.20–2.63 (m, 8H); 46.97; 49.82</td>
</tr>
<tr>
<td>20</td>
<td>66</td>
<td>153–155</td>
<td>(C_{19}H_{24}N_4O_4)</td>
<td>407(100)</td>
<td>408(26)</td>
<td>1019, 1035, 1132, 1383, 1395, 1439, 1466, 1714, 1769, 3322</td>
<td>2.42–2.85 (m, 8H); 37.87; 47.65; 48.79</td>
</tr>
</tbody>
</table>

\(^a\) The microanalyses were in good agreement with the calculated values: C ± 0.34, H ± 0.10, N ± 0.30;
\(^b\) by chemical ionization using methane as reactant gas. Therefore, for Mol. wt. M\(^*\) + 1 values are reported. Numbers in the brackets represent relative intensities of the peaks;
\(^c\) only shifts of methylene (–CH\(_2\)–) carbon atoms are presented.

In conclusion, the use of Nefkens’ reagent for the protection of primary amino groups in polyamines without affecting the secondary amino groups presents a simple and effective one-step procedure based on readily available and inexpensive commercial starting materials.

**Experimental**

**Materials**

All reagents were of the finest quality available commercially. Triethylamine was kept over potassium hydroxide pellets. N-Ethoxycarbonylphthalimide (the Nefkens’ reagent) was purchased from Fluka AG.

**Analytical procedures**

Melting points were determined on a Thomas Hoover melting point apparatus, model 6406-K with a calibrated thermometer. The IR spectra were recorded either on a Perkin-Elmer Spectrophotometer, model 735B or on a Nicolet-10MX FTIR Spectrophotometer. \(^1\)H NMR analyses were performed on a Varian 60 MHz NMR Spectrometer, model EM-360L. \(^{13}\)C NMR spectra were obtained on a Varian CFT-20 Spectrometer using an 8K data table, TMS standard, D\(_2\)O lock, and a sweep width of 4000 Hz. Mass spectra in the chemical ionization (CI) and/or electron impact (EI) modes were recorded on a Hewlett-Packard Mass Spectrometer, model 5985GS. Microanalyses were obtained on a Perkin-Elmer 240C Elemental Analyzer. TLC analyses were performed on silica gel 60F\(_254\) precoated sheets (EM Reagents), layer thickness 0.2 mm with visualization using UV light and/or iodine chamber.

**Preparation of \(N^a,N^o\)-bis(phthaloyl)polyamines 16–20**

General Procedure: A solution of N-ethoxycarbonylphthalimide (15) (2.19 g, 10.0 mmol) in chloroform (10 ml) was added rapidly at 20 °C to a solution of an appropriate polyamine 1–5 (5.0 mmol) in chloroform (10 ml). The resultant clear solution was kept at 20–25 °C for 45 min. Removal of the solvent on a rotating evaporator at
30 °C/20 torr gave the crude products either as solids or oils. Analytically pure products 17 and 18 were obtained by trituration of the crude solids with boiling methanol (10 ml). Analytically pure 16, 19 and 20 were obtained by crystallization of the oily crude products from methanol. The analytical data for the new compounds are presented in Table I.

Reaction of diethylenetriamine (3) with three equivalents of the Nefkens' reagent

A solution of diethylenetriamine (3, 0.52 g, 5.0 mmol) in chloroform (10 ml) was added rapidly to a stirred solution of the Nefkens' reagent (15, 3.28 g, 15.0 mmol) in chloroform (15 ml). The reaction mixture was stirred for 15 min at 25 °C and monitored by TLC (chloroform:methanol = 10:1, v/v) revealing basically two components 18 and the unreacted Nefkens' reagent. The reaction mixture was subsequently boiled with reflux (60 °C) for 30 min. At the end of this period it was found by TLC analysis that the mixture contained mainly 18 and the unreacted Nefkens' reagent, plus some minor impurities.

The reaction mixture was concentrated at 30 °C/20 torr to a volume of 15 ml. Boiling methanol (50 ml) was added, and the resultant solution was kept overnight in a refrigerator at 0 °C. The precipitated product was collected by filtration and washed with cold (0 °C) methanol (2×3 ml) to give 1.15 g (63%) of a pure compound, m.p. 178–180 °C. This product was identical in all respects with an authentic sample of 18 (Table I).

Preparation of N1,N7-bis(phthaloyl)-N4-valeroyl-1,5,9-triazanonane (21)

To a solution of 17 (1.17 g, 3.0 mmol) and triethylamine (0.50 g, 5.0 mmol) in dichloromethane (10 ml) was added dropwise, at −20 °C, a solution of valeroyl chloride (0.39 g, 3.2 mmol) in dichloromethane. The reaction mixture was stirred at 20 °C for 12 h. After dilution with 25 ml of dichloromethane the reaction mixture was washed with a 5% aqueous solution of citric acid (2×25 ml), water (25 ml) and a 2% aqueous sodium carbonate solution (2×25 ml). The organic layer was dried with anhydrous magnesium sulfate and filtered. Removal of the solvent on a rotating evaporator at 30 °C afforded a colorless, oily residue which crystallized slowly. Trituration of this material with a mixture of t-butyl methyl ether and n-hexane (1:1, v/v) followed by filtration gave 1.18 g (82%) of pure 21, m.p. 82–84 °C.

MS (CI): m/e = 476 (M+ + 1, 100%), 477 (M+ + 2, 32%). - 1H NMR (CDCl3): δ = 0.82 (t, 3H), 1.00–2.37 (m, 10H), 3.03–3.77 (m, 8H), 7.40 ppm (s, 8H). - 13C NMR (CDCl3): δ = 3.15–3.87 (m, 8H); 6.38–6.80 (m, 5H); 7.20 ppm (b.s., 8H). - 1H NMR (CDCl3): δ = 35.77 ppm (signal related to polyamine carbon atoms).

C24H36N6O6 (467.46)
Found C 69.71 H 4.74 N 9.04.

Preparation of N1,N2-bis(phthaloyl)-N4-phenylaminocarbonyl-1,4,7-triazaheptane (22)

To a solution of 18 (1.09 g, 3.0 mmol) and triethylamine (0.40 g, 4.0 mmol) in dichloromethane (25 ml) was added dropwise at 20 °C a solution of benzoyl chloride (0.46 g, 3.3 mmol) in dichloromethane (5 ml). After 2 h of stirring at 20–25 °C the reaction mixture was washed with 1N hydrochloric acid (2×50 ml), water (50 ml) and a 2% aqueous sodium carbonate solution. The organic layer was dried over anhydrous magnesium sulfate and filtered. Concentration of the filtrate on a rotating evaporator at 30 °C/20 torr gave the crude product 22. Crystallization of 22 from 90% aqueous methanol resulted in 1.02 g (73%) of pure 22, m.p. 135–137 °C.

C27H39N2O5 (496)
Ca. C 71.59 H 6.15 N 8.84.
Found C 71.86 H 6.13 N 8.80.

Preparation of N1,N2-bis(phthaloyl)-N4-phenylaminocarbonyl-1,4,7-triazaheptane (23)

Phenyl isocyanate (0.25 g, 2.1 mmol) was added in one portion to a boiling solution of 18 (0.73 g, 2.0 mmol) in dichloromethane (25 ml). After boiling with reflux for 30 min the reaction mixture was concentrated on a rotating evaporator at 30 °C/20 torr. Trituration of the solid residue with hot methanol (10 ml) followed by filtration, and washing of the solid with methanol (5 ml) afforded 0.94 g (97%) of the pure 23, m.p. 244–246 °C (dec). An analytical sample was prepared by crystallization of 23 from a mixture of dimethylformamide and n-hexane.

MS (CI): m/e = 496 (M+ + 1, 100%). - 1H NMR (CDCl3): δ = 0.82 (t, 3H), 1.00–2.37 (m, 10H), 3.03–3.77 (m, 8H), 7.40 ppm (s, 8H). - 13C NMR (CDCl3): δ = 3.15–3.87 (m, 8H); 6.38–6.80 (m, 5H); 7.20 ppm (b.s., 8H). - 1H NMR (CDCl3): δ = 35.77 ppm (signal related to polyamine carbon atoms).

C24H36N6O6 (467.46)
Found C 69.71 H 4.74 N 9.04.
3069 cm\(^{-1}\). - \(^1\)H NMR (CDCl\(_3\)): \(\delta = 1.34\) (b.s., 1H), 3.66 (t, 4H), 3.95 (t, 4H), 6.89–7.92 ppm (m, 13H). - \(^{13}\)C NMR (CDCl\(_3\)): \(\delta = 36.09, 45.25, 119.64, 122.70, 123.48, 128.59, 132.06, 134.10, 139.29, 154.88, 168.39\) ppm.

\[\text{C}_{27}H_{22}N_{4}O_5\] (482.48)

Calcd C 62.19 H 4.75 N 11.73.

Found C 62.64 H 4.75 N 11.73.

**Preparation of \(N^1, N^7\)-bis(phthaloyl)-\(N^4\)-benzoyl-1,4,7-triazahexane (26)**

To a solution of 20 (0.61 g, 1.5 mmol) and triethylamine (1.50 ml, 10.6 mmol) in dichloromethane (30 ml) was added dropwise a solution of benzoyl chloride (0.44 g, 3.1 mmol) in dichloromethane (5 ml) at 15–20 °C. After stirring for 2 h the reaction mixture was washed with 2 N hydrochloric acid (2×25 ml), water (25 ml) and a 2% sodium bicarbonate solution (50 ml). The organic layer was dried with anhydrous magnesium sulfate and filtered. Concentration of the filtrate on a rotating evaporator at 30 °C/20 torr gave the crude product 26 in the form of a white solid. Crystallization from 80% aqueous methanol gave 0.85 g (92%) of pure 26, m.p. 208–210 °C.

MS (EI): \(m/e = 615\) (M\(^+\) + 1, 36%), 391 (M\(^+\) – 223, 80%), 149 (100%). - IR (KBr): \(\nu = 725, 1110, 1150, 1265, 1415, 1650, 1775, 2920\) cm\(^{-1}\). - \(^1\)H NMR (DMSO-d\(_6\)): \(\delta = 2.30–3.00\) (m, 4H), 3.42–3.86 (m, 16H), 7.15–7.57 ppm (m, 12H), 133.91, 167.56, 172.27, 175.89 ppm.

\[\text{C}_{36}H_{30}N_{6}O_{10}\] (878.91)

Calcd C 66.96 H 5.11 N 9.25.

Found C 66.56 H 5.11 N 9.25.

**Preparation of \(N^1, N^{10}\)-bis(phthaloyl)-\(N^4, N^7\)-benzoyl-1,4,7,10-tetraazadecane (27)**

To a boiling solution of 22 (0.663 g, 1.42 mmol) in anhydrous ethanol (10 ml) was added, in one portion, a solution of anhydrous hydrazine (0.095 g, 2.98 mmol) in anhydrous ethanol (2 ml). The reaction mixture was maintained at reflux for 3 h, followed by removal of the solvent on a rotating evaporator at 40 °C/20 torr. The semi-solid residue was dissolved in water (15 ml), and the solution was acidified with acetic acid (1 ml). The precipitated 2,3-dihydro-1,4-phthalazinedione was collected by filtration and the filtrate concentrated on a rotating evaporator at 60 °C/20 torr. The oily residue was dissolved in ethanol (10 ml), and the solution mixed with a solution of oxalic acid dihydrate (0.447 g,
3.55 mmol, 25% excess) in ethanol (25 ml). The instantly formed white precipitate was collected by filtration and washed with ethanol (2×5 ml) and ethyl ether (2×5 ml). Recrystallization of the crude product (0.595 g) from water gave 0.472 g (86%) of pure preparation.

**Preparation of \( N,N,N^1,N^2\)-tetra(3-aminopropyl)-glutaryl diamide tetraoxalate (28)**

A solution of hydrazine hydrate (100%, 0.210 g, 4.2 mmol) in anhydrous ethanol (3 ml) was added rapidly to a boiling suspension of 25 (0.880 g, 1.0 mmol) in anhydrous ethanol (17 ml). The stirred reaction mixture was boiled with reflux for 4 h. After removal of the solvent on a rotating evaporator at 40 °C/20 torr, the residue was dissolved in water (50 ml) and acidified with oxalic acid dihydrate (0.63 g, 5.0 mmol). The white precipitate of 2,3-dihydro-1,4-phthalazinedione was collected by filtration and washed with hot water (2×10 ml). Concentration of the combined washings and filtrate on a rotating evaporator at 60 °C/20 torr gave the crude preparation. Crystallization from aqueous methanol afforded 0.485 g (67%) of pure 28, m.p. 182–184 °C (dec.). Mass spectrum could not be recorded because the sample did not vaporize.

**IR (KBr):** 

IR (KBr): \( \nu = 1228, 1280, 1385, 1404, 1559, 1637, 1703, 1720, 2150–3050 \text{ cm}^{-1} \). 

**\( ^1H\) NMR (D2O/NaOH):** \( \delta = 2.50 (t, 4H), 3.16 (t, 4H), 6.75–7.26 \text{ ppm (m, 5H)} \). 

**\( ^13C\) NMR (D2O/NaOH):** \( \delta = 39.41, 40.09, 47.44, 50.65, 127.13, 128.88, 132.21, 133.66, 170.68 \text{ ppm} \).

**C25H46N6O18** (718.68) 

Calcd C 41.46 H 6.07 N 11.50. 

Found C 41.78 H 6.45 N 11.69. 

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